THE

Malayan Agricultural Journal.

Vol. XVI. MARCH, 1928. No. 3.

Studies on Tapioca.

V. R. GREENSTREET.

An investigation carried out at the Department of Agriculture of the impoverishment of the soil by tapioca necessitated periodic analyses of the plant. This called for an investigation of methods of drawing a representative sample of a standing crop, computing the total weight of the crop by sampling and of rapid methods of carrying out the chemical analysis of a large number of samples.

The results of investigations on these lines are described in the two following articles which are intended to be the first of a series dealing with chemical aspects of the cultivation and manufacture of tapioca.

I. SAMPLING A STANDING CROP.

As a preliminary experiment sample plants were drawn at the time of harvesting from Block 29, a variety trial at Serdang Experimental Plantation. Each plot consisted of three rows of twenty-two plants each, the spacing being 3 x 3 feet. The centre plant of the centre row of each plot was taken and the tuber, stem and leaf weighed separately. From the total weight deduced from the samples and the actual weight of tubers obtained when the plots were harvested the P.Em in sampling was calculated. These figures showed the number of plants of each variety which would need to be sampled to give a P.Em of not more than 5 per cent. The results are recorded in Table 1.

TABLE I.

Comparison between actual weight of harvest and weight calculated from samples.

Variety (G.E.P.S. ref.)	Number of plants sampled.	Harvesting	Sampling	Calculated number of plants that should be sampled to give an accuracy of 5 per cent.
		Actual error per cent on weight of harvest.	P. Em. per cent.	
Native 1 ...	20	14	7	40
Native 2 ...	8	1	10	33
Native 3 ...	8	15	13	47
Phillipine 1 ...	8	15	2	4
Phillipine 2 ...	8	4	12	48
Phillipine 3 ...	8	21	4	7
Phillipine 4 ...	7	5	9	24
Phillipine 5 ...	8	25	6	11
Java 6 ...	8	14	7	15
Java 7 ...	8	4	7	19
Java 8 ...	8	4	11	43
Java 9 ...	7	4	8	20
Java 10 ...	7	2	12	40

The divergence exhibited by these results appears to indicate that the tendency to vary is much greater in some varieties than in others. In the case of variety Native No. 1, the subject of the main experiment, it appears to be necessary to collect at least 40 plants in order to attain an accuracy of 5 per cent.

The main experiment was carried out on Block 30, a manurial trial at Serdang Experimental Plantation, which consisted of 20 half-acre plots. The theoretical stand per plot was 2442 in 33 rows of 74 plants each. In plots A

and B the diagonal method of sampling was employed and it was found convenient to take 33 plants per sample viz. one plant per row. This method of sampling was compared with a chequerboard method whereby the plot was divided into 33 equal rectangles the same shape as the plot and the centre plant of each rectangle taken. The whole crop was harvested on the following day. Owing to the difficulty of harvesting the leaves alone the whole of the "tops" were harvested and weighed; after quartering, the leaves (including leaf stem, flower and flower stem, fruit and fruit stem) were separated from the stalk and the total amount calculated. The error in sampling is shown in Table II.

TABLE II.

Plots A. & B.

Difference between actual total yield of tubers, stem and leaf and that computed by sampling 33 plants diagonally and chequerboard.

	Missing Plants.		Sampling diagonally.	Sampling chequer-board.
	Number.	Per cent.	Error per cent. on actual weight of sampled crop.	Error per cent on actual weight of sampled crop.
Plot A 1.	204	8.4		
Tuber			+ 17	+ 14
Stem			+ 14	+ 8.5
Leaf			+ 31	+ 31
Plot B 1.	246	10.0		
Tuber			+ 9.2	- 7.8
Stem			+ 3.3	- 9.5
Leaf			Nil	Nil
Plot A 2.	232	9.5		
Tuber			+ 11	+ 2.9
Stem			+ 11	+ 10
Leaf			- 13	- 13
Plot B 2.	79	3.2		
Tuber			+ 18	+ 23
Stem			+ 5.5	+ 16
Leaf			Nil	Nil

The harvested leaf, stem and tuber were sampled by continued quartering and the leaves of the diagonal and chequerboard samples similarly treated. In order to determine whether the chemical composition of the sampled material was representative of the harvest nitrogen determinations were carried out on the leaf, the results being shown in Table III.

TABLE III.

Plots A. & B.

Comparison between results of nitrogen determinations on harvested and sampled tapioca leaf.

Plot.	Harvested.	Sampled.			
		Diagonal.		Chequerboard.	
	Nitrogen Per cent.	Nitrogen per cent duplicates.		Nitrogen per cent duplicates.	
A 1 -	1.25	1.06	1.11	0.89	0.92
B 1 -	1.00	1.01	1.02	1.07	1.08
A 2 -	1.04	0.90	1.13	1.13	1.20
B 2 -	1.09	0.98	1.28	1.04	1.12

The results of nitrogen determinations show differences between duplicates amounting to 30 per cent. This discrepancy is probably attributable to the difference in hardness and density of the leaves and seeds militating against thorough mixing of two fractions of dissimilar composition.

In plots C and D the diagonal method of sampling was compared with a method devised by Sir John Russell for sampling at different stages of growth and described by him as follows:—

> " Divide the plot lengthwise into a number of strips,
> " each not less than three rows wide. Also divide it

" transversely into as many strips as there are sampl-
" ings to be made. For the first sampling, decide by
" random selection which squares shall be taken, one
" from each vertical row, then collect a suitable num-
" ber of plants at intervals from within each square.
" Guarded plants only must be taken, a "guarded"
" plant being one from an inner row of the square
" with no gaps on any side of it. For the second
" sampling, decide at random which of the remaining
" squares shall be sampled, again taking one from
" each vertical row, and proceed as before. Repeat
" the method for the remaining samplings."

Each plot under investigation was divided lengthwise into eleven strips of three rows each and transversely into twelve strips of six rows each, so that if necessary the method could be used for taking monthly samples over a period of one year. To be comparable with the diagonal method three plants were taken from each square. The process of division into strips was somewhat complicated but the principal difficulty arose in the avoidance of unguarded plants since approximately 10 per cent. were missing. The method of taking the sample is shown diagramatically in figures 1, 2, 3, and 4. It will be observed that owing to gaps only half the plants actually earmarked for sampling could be taken thus requiring plants adjacent to other squares or in many cases plants actually in other squares to be taken. As a result it would have been impossible to collect twelve monthly samples. Table IV shows the error in sampling.

TABLE IV.

Plots C. & D.

Difference between actual total yield of tuber, stem and leaf and that computed by sampling 33 plants diagonally and by Russell's method.

	Missing Plants.		Sampling Diagonally.	Sampling by Russell's Method.
	Number.	Per cent.	Error per cent. on weight of sampled crop.	Error per cent on weight of sampled crop.
Plot C1. -	201	8.2		
Tuber -			+ 14	+ 3.7
Stem -			- 10	- 21
Leaf -			- 22	- 16
Plot D 1.	211	8.6		
Tuber -			+ 1.3	- 2.6
Stem -			+ 6.3	- 5.6
Leaf -			- 9	- 12
Plot C 2. -	213	8.7		
Tuber -			+ 5.3	+ 12
Stem -			+ 4.5	+ 17
Leaf -			- 21	+ 9.3
Plot D 2. -	219	9.0		
Tuber -			Nil	+ 20
Stem -			- 11	+ 13
Leaf -			- 43	- 27

The procedure on harvesting and sampling was the same as that previously described except that moisture, ash and nitrogen determinations on tuber both sampled and harvested were carried out. The results of analysis are shown in Table V.

TABLE V.

Plots C. & D.

Comparison between results of moisture, ash and nitrogen determinations on harvested and sampled tapioca tuber.

Plot.	Origin of Material.		Moisture per cent in duplicate.	Ash per cent in duplicate.	Nitrogen per cent in duplicate.
C1.	Sampled.	Russell's method	63.0	1.31	0.26
			65.1	1.08	0.30
		Diagonally	63.2	1.18	0.26
			64.4	1.36	0.24
	Total harvest		61.9	1.29	0.27
			64.5	1.42	0.28
D1.	Sampled.	Russell's method	65.1	1.87	0.31
			65.2	1.81	0.25
		Diagonally	63.4	1.58	0.28
			63.8	1.78	0.28
	Total harvest		62.8	2.25	0.23
			63.7	2.23	0.26
C.2	Sampled.	Russell's method	63.2	1.14	0.23
			62.4	1.21	0.26
		Diagonally	64.3	1.10	0.27
			64.4	1.09	0.27
	Total harvest		60.7	1.21	0.25
			...	1.25	0.27
D2.	Sampled.	Russell's method	63.2	1.24	0.24
			64.2	1.23	0.26
		Diagonally	63.0	1.23	0.24
			63.8	1.28	0.24
	Total harvest		64.4	1.25	0.24
			64.0	1.26	0.24

The results of nitrogen determinations show that a much closer agreement between duplicates can be obtained with tuber than leaf. Ash determinations on duplicate samples however show less agreement than nitrogen determinations; this lack of agreement was suspected of being due to adhering soil and insufficient fineness of chopping. When dealing with plots C2 and D2 therefore the cleaning was more thorough and the fineness of chopping increased with the result that discrepancies between duplicates were reduced and agreement all round improved.

Since no one method of sampling gave an error consistently smaller than any other yet the mean of four plots gave an error of about 5 per cent, plots E, F, G, H, I and J were sampled diagonally to give 132 plants per sample by taking two diagonals and two plants per row per diagonal per plot.

It having been shown in table V that the chemical composition of the sampled plants was in agreement with that of the harvested material samples for analysis were drawn only from the diagonal samples. In consequence of this leaves and stem were not harvested separately but bulked as "tops". Table VI shows the error in sampling in plots E, F, G, H, I and J.

TABLE VI.

PLOTS E, F, G, H, I, J.

Difference between actual total yield of tuber and tops and that computed by sampling 132 plants diagonally.

	Missing Plants.		Sampling diagonally error per cent on weight of sampled crop.
	Number.	per cent.	
Plot E 1 -	253	10.4	
Tuber - -			+ 4.9
Tops -			− 5
Plot F 1 -	639	25.2	
Tuber -			+ 24
Tops -			+ 19
Plot E 2 -	212	8.7	
Tuber -			+ 7.7
Tops -			nil.

	Missing Plants.		Sampling diagonally error per cent on weight of sampled crop.
	Number.	per cent.	
Plot F 2 -	369	15.0	
Tuber -			+ 9.7
Tops -			− 7.5
Plot G 1 -	166	6.8	
Tuber -			+ 3
Tops -			− 8.7
Plot H 1 -	235	9.6	
Tuber -			+ 3.4
Tops -			− 10
Plot G 2 -	117	4.8	
Tuber -			+ 21
Tops -			+ 7.1
Plot H 2 -	214	8.8	
Tuber -			− 18
Tops -			− 7.3
Plot I 1 -	387	15.8	
Tuber -			+ 16
Tops -			+ 8.1
Plot J 1 -	485	19.8	
Tuber -			+ 5.6
Tops -			− 8.5
Plot I 2 -	335	13.7	
Tuber -			+ 14
Tops -			+ 18
Plot J 2 -	597	24.4	
Tuber -			+ 19
Tops -			+ 6.6

In determining the mean error of sampling for the various methods it was considered desirable to exclude plots which by reason of excessive numbers of vacancies were likely to be unrepresentative. The theoretical stand per half acre plot was 2442 plants and therefore in calculations of mean error of sampling, plots containing less than 2100 plants, equivalent to 86 per cent. (Plots F1, F2, I1, J1 and J2) were excluded. Table VII shows the mean error of sampling by the various methods.

TABLE VII.

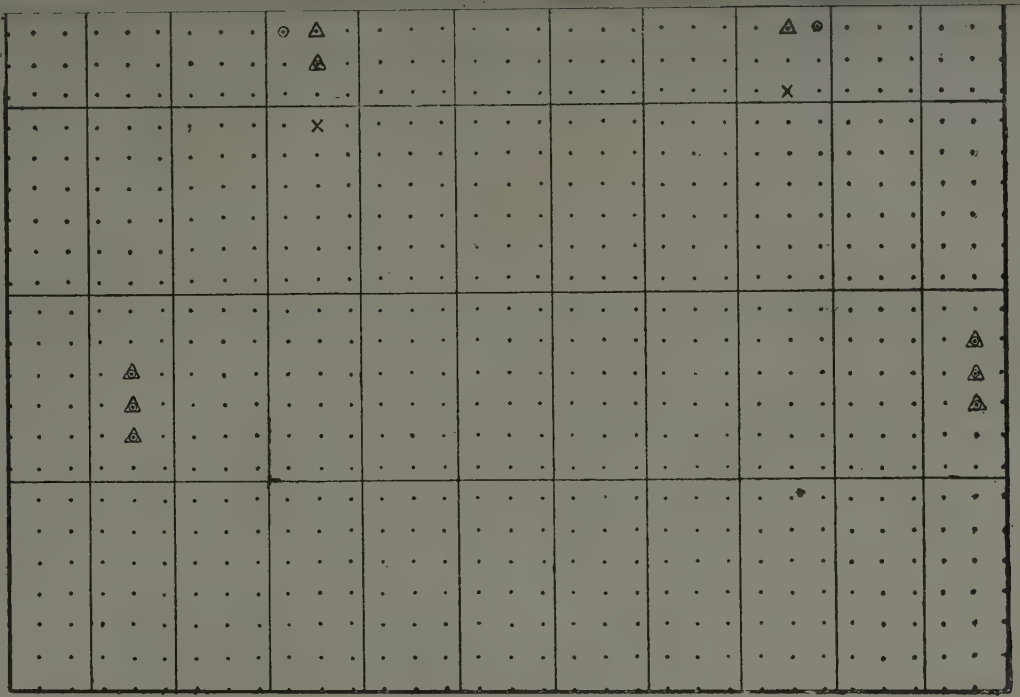
Comparison between mean error of sampling by various methods.

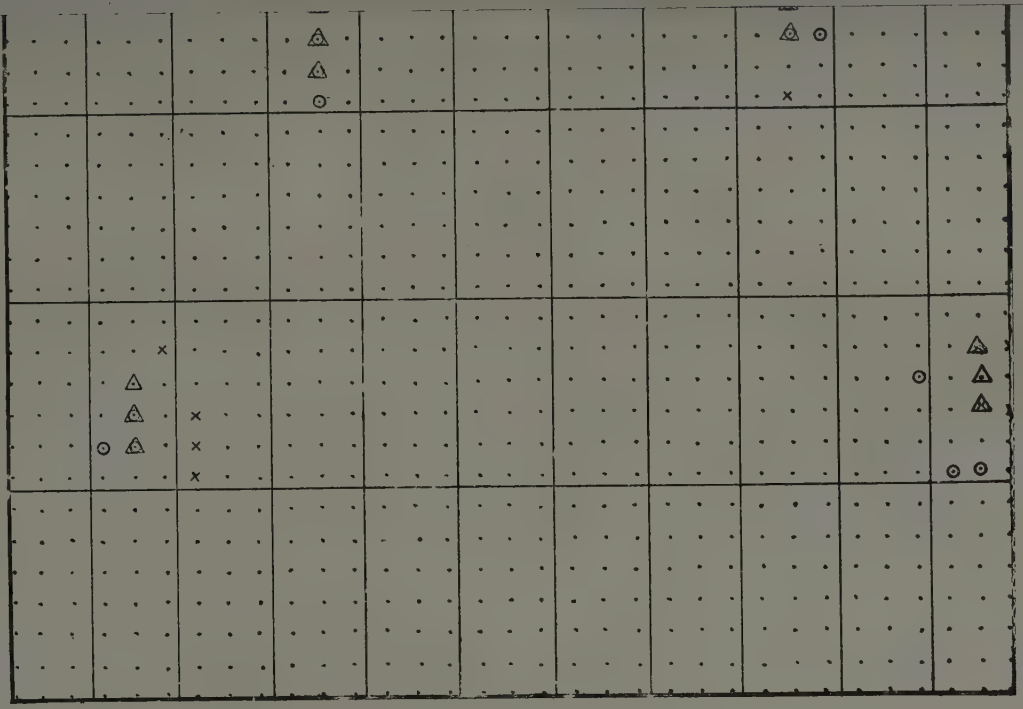
Method of Sampling.	Number of plants in sample.	Number of plots sampled.	Error per cent Plus or Minus.			
			Tuber.	Leaf.	Stem.	Tops.
Diagonal -	33	8	9.5	17	8.2	...
Chequerboard	33	4	12	11	11.0	...
Russell -	33	4	9.5	16	17	...
Double Diagonal -	132	7	10.	...	...	8.0

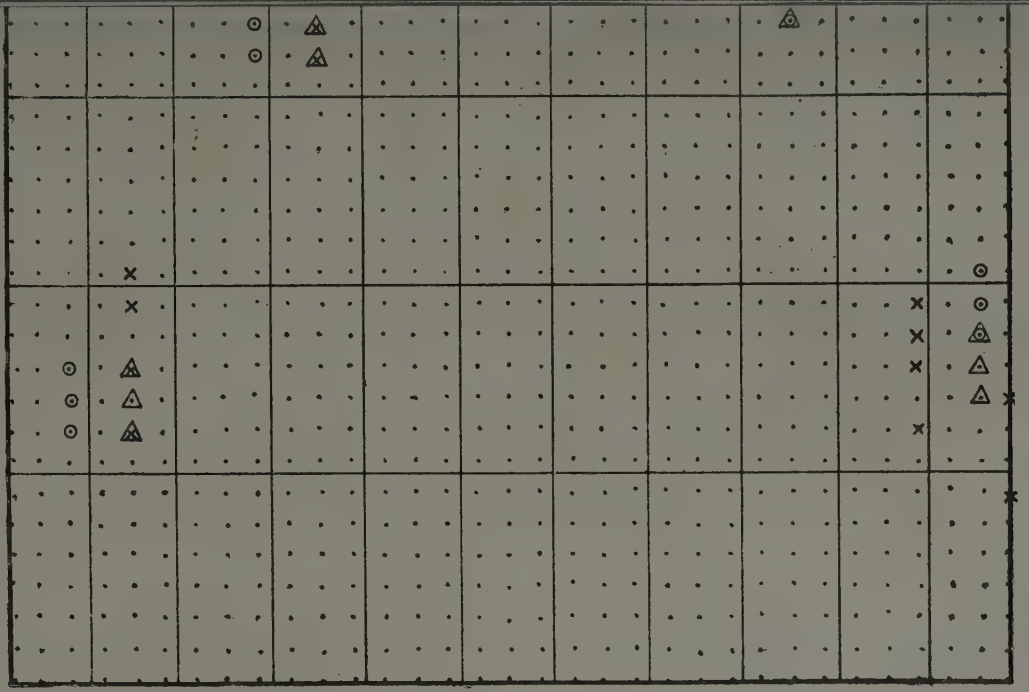
It appears from the figures recorded in Table VII that there is no very significant difference in accuracy between 33 plant samples taken by the diagonal, chequerboard or Russell's method. According to Russell's method the mean of four plots gives an error of sampling tuber and leaf approximately equal to that obtained by the mean of eight plots sampled diagonally. The error in sampling stem in the case of eight plots sampled diagonally is only half that obtained by sampling four plots by Russell's method. By chequerboard sampling four plots the mean error in the weight of tuber is greater than that obtained by either of the other methods but shows close agreement with that of the error in weight of leaf and stem. Turning to a consideration of the seven plots sampled by the method of taking 132 plants in double diagonals it is evident that no improvement in accuracy of sampling can be obtained by increasing the number of plants in the sample beyond 33 and by sampling it is practically impossible to obtain an accuracy greater than 10 per cent.

Conclusions.—When sampling a standing crop of tapioca planted 3 x 3 feet provided there are not more than 14 per cent. vacancies the yield of tubers and "tops" may be computed to within 10 per cent. by taking a sample of 33 plants per half acre.

The plants may be taken by either Russell's method, diagonally or chequerboard.







The chemical composition of such a sample is representative of that of the crop.

The method devised by Sir John Russell although more complicated has the advantage that provided the number of vacancies is not excessive it may be employed for repeated sampling at progressive stages of growth.

Received for publication 25th April, 1928.

II.—The Coeruleo-Molybdate Method for Determination of Phosphates.

The blue coloration produced by the addition of a phosphorus compound followed by a reducing agent to a solution containing ammonium molybdate and stannic chloride was investigated by Denigés[1]. He proved that it was a phospho-molybdenum compound and could exist only in the presence of a mineral acid whereas the similar blue colour formed by the interaction of ammonium molybdate and stannic chloride was a basic oxide of molybdenum and discharged by mineral acids. Another distinguishing feature was that the non-phosphorus blue was insoluble while the phosphorus blue was soluble in ether. Denigés found that 0.2 m.g. of phosphoric acid in 5 cc of solution was sufficient to produce a blue colour even in the presence of organic matter such as blood, urine or milk and that while care had to be exercised to ensure sufficient acidity to prevent non-phosphorus blue from appearing, the sensitiveness of the reaction was reduced by an excess of mineral acid or molybdate. Denigés further discovered that while arsenic reacted similarly to phosphorus, silica, fluorine or boron hindered or prevented the reaction.

The method was subsequently employed by Atkins[2] for the analysis of solutions containing between 0.05 and 0.50 gms. of phosphoric acid per million and described by him as follows:—

> " To 100 ccs of solution add 2 ccs of Reagent A (100
> " ccs of 10 per cent. ammonium molybdate and 300
> " ccs of 50 per cent. by volume sulphuric acid and
> " stored in the dark) and 5 drops of freshly prepared
> " Reagent B (0.1 gm. of tin, one drop of 4 per cent.
> " copper sulphate and 2 cc concentrated hydrochloric
> " acid made up to 10 cc). Treat 100 cc of standard
> " phosphate solution similarly and compare the tint.
> " The blue colour reaches its maximum intensity in
> " five minutes. On standing and especially when ex-
> " posed to the light Reagent A spontaneously develops
> " a blue colour which however may be allowed for by

[1]Denigés. Compt. Rendu. 1920. 171, 802.
[2]Atkins. J. Ag. Sc. 1921, 16, 192.

" carrying out a blank estimation. In the event of a " greenish tint occuring this may be counteracted by " the addition of a little Bismarck Brown."—

Parkes[3] used the method for the determination of phosphorus in aqueous soil extracts. He used a solution containing not more than 0.3 parts per million phosphoric acid made up to 97.5 cc: reagents A and B were added and after standing for 10 minutes the tint compared with the standard in Nessler tubes. Compared with the volumetric method, Parkes obtained an accuracy of about 2 per cent.

Gilbert and Smith[4] have shown how the colour may be inhibited by acids and intensified by bases.

In view of the satisfactory results obtained by these investigators it was proposed to use the method in soil analysis. Determinations were consequently carried out to determine the effect on the accuracy of the method of some of the other constituents of the soil.

Experimental.

A standard phosphate solution containing one part phosphoric acid per million was prepared. It was just possible to put in correct order the blue colour produced by amounts of 5, 10, 15, 20, 25 and 30 cc of standard solution in Nessler tubes. As it was obviously necessary to attain a greater degree of accuracy than this Kobers colorimeter was employed for all the following determinations.

Determination of accuracy.—A 1.5 per cent. solution of copper sulphate was compared with a 3 per cent solution. The colorimeter result gave a ratio of 100: 198. The accuracy of reading was therefore 1 per cent.

Since after removing silica the two preponderating elements were aluminium and iron determinations were carried out on solutions to which had been added amounts of these elements approximately equivalent to the proportions found to be present in samples of Serdang soil.

[3]Parkes, Soil Sc. 1925, 20, 149.
[4]Gilbert and Smith J. Biol Chem. 1927, 74, 223.

Determination of the effect of iron.—0.001 gm. of ferric chloride when added to 30 cc of standard phosphate solution had no appreciable effect on the tint. Determinations using Kobers colorimeter were carried out. The best tint for consistent readings was found to be that given by 50 cc standard phosphate solution diluted to 100 cc and with the colorimeter tubes at 30 on the scale. 50 cc of standard phosphate solution containing .00005 gm P_2O_5 and made up to 100 cc was compared with a similar solution containing 5 cc of N/100 ferric chloride i.e. .00075 gm of iron or about 15 times as much iron as phosphorus. The colour was developed in the colorimeter tubes, stannic chloride reagent being added drop by drop until no further deepening of the tint occurred. Readings were taken every minute and the result calculated from the maximum depth of tint observed. The presence of ferric iron in the proportion Fe_2O_3 : P_2O_5 : : 15 : 1 gave a reading 6 per cent. too low.

Determination of the effect of Aluminium.—50 cc of standard phosphate solution was made up to 100 cc and compared with a similar solution containing 0.1 cc of 10 per cent. potash alum. The determination was carried out in the same way as before. The effect of the presence of aluminium in the proportion Al_2O_3 : P_2O_5 : : 100 : 1 was nil.

Determination of the combined effect of Iron and Aluminium.—50 cc of standard phosphate solution was compared with a similar solution containing 5 cc of N/100 ferric chloride and 0.1 cc of 10 per cent. potash alum. The presence of iron and aluminium in the proportions Al_2O_3 : Fe_2O_3 : P_2O_5 : : 100 : 15 : 1 gave a reading 2 per cent. too high.

Determination of the effect of stale stannic chloride (Reagent B)—One day old stannic chloride (Reagent B) gave a tint 1 per cent. deeper than the freshly prepared solution.

It appears from these results that the presence of iron and aluminium in the proportions Al_2O_3 : Fe_2O_3 : P_2O_5 : : 100 : 15 : 1 have very little effect on the result while the use of stannic chloride reagent one day old gives a result within the limits of the experimental error. Since at this time the attention of the writer was diverted to the analysis of the tapioca plant comparative estimations of phosphoric acid in it by the coeruleo-molybdate and gravimetric methods were proceeded with without any further preliminaries.

Comparison of Coeruleo-Molybdate with Gravimetric method.—The following results for the percentage of phosphoric acid in various substances were obtained by the coeruleo-molybdate and gravimetric methods respectively, all determination being carried out on silica-free hydrochloric acid solutions.

TABLE I.

Results of determinations of phosphoric acid by Coeruleo-molybdate and Gravimetric methods in silica free hydrochloric acid solutions.

Substance.	Phosphoric Acid (P_2O_5) per cent.		Error on B per cent.
	Gravimetric A	Colorimetric B	
Tapioca tuber			
4-6-26	0.19	0.17	− 10
31-3-27	0.24	0.22	− 9
29-4-27	0.24	0.23	− 8
30-5-27	0.24	0.25	+ 4
29-6-27	0.22	0.25	+ 11
11-8-27	0.24	0.27	+ 11
Tapioca stem			
4-6-26	0.12	0.13	+ 8
31-3-27	0.33	0.27	− 22
29-4-27	0.26	0.25	− 4
30-5-27	0.29	0.30	+ 2
29-6-27	0.28	0.33	+ 16
11-6-27	0.26	0.28	+ 7
Tapioca leaf			
4-6-26f	0.22	0.25	+ 14
31-3-27	0.80	0.70	− 14
29-4-27	0.58	0.66	+ 12
30-5-27	0.55	0.63	+ 13
29-6-27	0.61	0.66	+ 7
11-8-27	0.45	0.65	+ 31
Oil palm leaflets			
B23-5-27	0.107	0.11	+ 2
H26-5-27	0.106	0.11	+ 4.5
S22-4-27	0.101	0.093	− 9
E	0.102	0.093	− 10
T	0.147	0.13	− 13
Serdang soil 20-1			
Surface	0.08	0.06	− 25
Control	0.09	0.07	− 22

The tapioca and oil palm solutions were rich in alkalis and alkaline earths while the soil solutions were rich in alumina and iron and contained traces of fluorine and silica. The control determination on Serdang soil was carried out by adding a known weight of phosphoric acid to the soil and subtracting it from the result.

It is evident that the solutions of tapioca stem and leaf contain substances which interfere with the colour reaction. There appear to be present one substance which accentuates the colour and another substance which is inhibitory.

Although the actual estimation is very rapid since it occupies only a few minutes for each colorimetric determination the elimination of silica is a lengthy process.

With a view to discovering whether the method could be rendered more rapid a series of determinations was carried out on the sulphuric acid solutions of the original substances without removing silica and prepared for nitrogen determinations by Kjeldahl's method.

TABLE II.

Results of determinations of Phosphoric acid by Coeruleo-Molybdate method in siliceous sulphuric acid solutions.

Substance.	Phosphoric Acid (P_2O_5) per cent.	Error compared with gravimetric per cent.
Tapioca stem		
31-3-27 -	0.28	- 18
29-4-27 -	0.21	- 21
30-5-27 -	0.22	- 34
29-6-27 -	0.27	- 4
11-8-27 -	0.18	- 48
Tapioca tuber		
31-3-27 -	0.22	- 9
29-4-27 -	0.17	- 46
30-5-27 -	0.16	- 48
29-6-27 -	0.20	- 10
11-8-27 -	0.23	- 5
Tapioca leaf		
31-3-27 -	0.50	- 60
29-4-27 -	0.54	- 7
31-5-27 -	0.61	+ 9
29-6-27 -	0.77	+ 20
11-8-27 -	0.46	+ 2
Oilpalm leaflets		
H 26-5-27 -	0.13	+ 17
Pineapple ash -	5.0	- 18

The results recorded in Table II indicate that the coeruleo-molybdate method is less accurate when carried out on solutions rich in acid and silica and thereby confirm Denigés' contention that silica may tend to prevent the reaction.

Conclusions.—The coeruleo-molybdate method for determination of phosphates in silica-free solutions as applied to tapioca tubers and oil palm leaflets gives results the maximum error of which is 13 per cent. and the mean error about 8 per cent. This is considered satisfactory since the error is within that involved in sampling in the field.

Applied to tapioca leaf and stem, pineapple ash and soil the coeruleo-molybdate method proves less satisfactory; an error as high as 31 per cent. being obtained in one instance.

When applied to more acid solutions rich in silica the coeruleo-molybdate method almost invariably gives low results[5], in one instance the result being only 40 per cent. of the true value. To determine the order of magnitude of the phosphate content of a material when nitrogen is to be estimated determination in "Kjeldahl's" solution may prove of use.

Received for publication 25th April, 1928.

[5]This agrees with the results obtained by Parker and Fudge (Soil Science 1927, 24, 109) but not seen until the completion of the greater part of this investigation.

Preliminary Report on Fusarium Cubense Causing Panama Disease in Malaya.

F. S. Ward.

Fusarium cubense, Erwin F. Smith, is the parasitic fungus which causes Panama Disease on the cultivated bananas, *Musa sapientum* and *Musa cavendishii.*

The Banana and Methods of Cultivation.—The banana is a large perennial herb, requiring from twelve to twenty months from time of planting to attain maturity. The cultivated bananas have small degenerate seeds which are usually functionless and useless for propagating. New plants are started by means of suckers that arise from the tuberous underground stem rhizome. These suckers are cut away from the parent plant when they are several months old. The top and roots are pruned off and the young "bulb" or "bit" is planted in an excavation made to receive it. The bulbs may be set out immediately or they may be allowed to dry for several days.

A banana plant produces only one bunch of fruit which is harvested by cutting down the whole stem, a sucker from the rootstock being allowed to take its place. The stem of the banana is a pseudostem, the outer surface of which consists largely of the remains of old leaf bases.

Symptoms of Panama Disease.

External.—Where Panama Disease has been present in a field for only a few months, several isolated cases of the disease are to be found; this indicates that the fungus has not yet become thoroughly and uniformly distributed in the soil, consequently the affected plants do not exhibit any external symptoms of the disease until they are approaching maturity. It is not uncommon to find a banana plant with a half formed bunch of fruit, the development of the bunch at that stage having been arrested by the death of the plant. In these mature plants, the symptoms are not unlike those brought about by severe drought. A typically diseased plant first shows a yellowing of the lower or outer leaf blades and petioles. The transition from the normal dark green colour of the leaf to a vivid yellow is usually sudden and proceeds from the margin inward.

Sometimes, one of the younger leaves shows signs of wilting while those below remain green. Usually, the oldest leaf is the first to go, then, in order, the next oldest and so on up to the topmost leaf, which invariably remains green the longest.

The first attacked leaves begin to wilt almost immediately. Within a day or two the fleshy leaf stalk buckles at a point three or four inches from the pseudostem and the leaf hangs pendant from this point.

In the case of young plants in abandoned fields where the disease is of long standing, the plants show a decided dwarfing effect.

A symptom which frequently accompanies stunting, is a longitudinal splitting of the outer leaf bases which form the pseudostems. The split may extend all the way from the rhizome, at the base or surface of the ground, to the collar or place where the leaves diverge, or it may be more limited in extent. Only the outer leaf bases may be involved, or the opening may extend to the centre of the pseudostem, in which case it may happen that the young leaf becomes diverted from its course up through the centre, and projects out through the split.

It is a curious fact that in any particular region this disease attacks most severely and sometimes exclusively the variety which is there most esteemed and therefore most widely planted.

The "Pisang Embun" variety in Malaya up to the present time appears to be most susceptible to the disease. The "Pisang Embun" is probably the most desirable and most widely grown variety of banana in Malaya. The "Gros Michel" variety which is grown extensively in Cuba, Panama, Costa Rica, Guatemala, Honduras and Jamaica is very similar to the "Pisang Embun."

Internal Symptoms of Panama Disease.—Healthy banana tissue, both in the rhizome and pseudostem is almost dead white when first cut open. After a few minutes, especially if it has been cut with a steel knife, a purplish discolouration will appear uniformly distributed over the cut surface, due to the presence of oxidising enzymes.

A cross section of a diseased rhizome shows on the surface small dots and irregular threads of a yellowish or light brown colour, either distributed evenly over the whole cut surface or arranged in a band just inside of the endodermis. Upon examination these dots and lines are made out to be the discoloured vascular bundles. Upon cutting successive sections of the rhizome towards the apex, it is found that the discolouration gradually becomes less pronounced in an upward direction and finally disappears altogether.

At an advanced stage of the disease, when most of the leaves are wilted, a cross section of the rhizome shows the vascular bundles to have changed in colour, from reddish brown to purple or even black, and most of the inner tissue is quite dark in colour.

With regard to the root system, numerous blackened roots, close to the bulb and extending into portions of the stele are frequently found.

Since 1908 when Panama disease first attracted wide attention in Panama and Costa Rica, the disease has appeared in many parts of the world.

The countries which have been affected include the Canaries, Jamaica, Honduras, the Philippines, Porto Rico, St. Lucia, Sierra Leone, Trinidad, South America and West Africa.

It is estimated that, in Panama, in 1910, at least 15,000 to 20,000 acres of banana plantations had been abandoned and many thousands more were seriously affected, while in Costa Rica the damage had been even greater.

The first record of Panama Disease being present in Malaya was in October, 1925, when Dr. O. A. Reinking observed in Singapore a banana wilt disease, but isolations showed that *Fusarium cubense*, the fungus causing Panama Disease, was not present.

In May 1927, in Johore, attention was drawn to a banana wilt disease on "Pisang Rastali" variety. After examining several cases there appeared to be two different types of disease present which could be recognized by their internal symptoms. These two diseases appear to be caused by two different organisms—(1) Bacterium (2) Fungus.

External Symptoms.—The external symptoms of these two diseases were practically the same and suggested the presence of Panama Disease, excepting that the longitudinal splitting of the outer leaf sheaths commonly found in true Panama Disease, was absent. Few external symptoms were exhibited by the affected plants until approaching maturity. In some cases, the disease was not apparent until after the fruit bunches had started to form. The lower external leaves start to wilt and turn yellow along the margin. This discoloration afterwards appears in blotches and changes to brown. The wilt gradually extends towards the mid rib. Often the wilted leaves are found hanging pendant while the central portions still remain green. Water soaked areas are often present at the leaf bases, but this applies more to the bacterial disease than to the fungus disease. The tops of the central leaves are sometimes found to be wilting before unfurling, but this symptom is present in the more severe cases of both of these diseases.

Bacterial Infection?

Internal Symptoms.—A transverse section of a diseased rhizome shows a yellowish white exudate oozing from the exposed surfaces. About half way between the centre and the periphery of a transverse section, a black ring will be seen, which is usually the only indication of decay within an affected plant (see photograph E). The exposed surfaces blacken rather rapidly on exposure to air for a few minutes. There is no sign of red or brown discoloured vascular bundles either in the central or peripheral parts. A longitudinal section of an affected plant shows the central black ring-rot more intensified towards the upper portion of the pseudostem than towards the base. The infection appears to originate in the bud-tissue. There is no indication of a decay extending into the internal tissue of the rhizome from the roots. A short bacillus was isolated from the gummy exudate; the cultural characteristics of this organism resemble those described by S.F. Ashby for the bacterium isolated from bananas affected with a wilt disease in Trinidad. Inoculations, however, with the Jchore organism have proved unsuccessful. This may be explained by the fact that this organism was being cultured in meat extract agar several weeks before the inoculations were carried out, owing to the delay in obtaining the required variety of banana. It was also necessary to allow the rhizomes to make suitable

growth before the inoculations were performed. It is possible that the organism lost considerable virulence before being used for inoculation purposes.

Fungus Infection?

Internal Symptoms.—In the case of the affected rhizomes from which the *Fusarium* fungus was isolated, there is not the same kind of gummy exudate present on the surface of a freshly cut section as described for the bacterial infection above. A transverse section near the base of a sucker affected with this type of disease shows a central heart rot throughout the pseudostem (see photograph C). An exudation oozes out, which has a more liquid and transparent consistency. A similar exudation is found on the surfaces of freshly cut sections taken from healthy rhizomes.

This central heart rot of the pseudostem, as in the previous type of disease, does not show any discoloration of vascular bundles within the tissue bordering the area where the decay exists. The roots appeared to be healthy.

The Fusarium fungus which was isolated from the diseased tissue closely agrees with *F. cubense* in form and in culture.

Two series of preliminary inoculations were carried out as follows. The wounds were wrapped around with sterilized cotton wool.

Bacterial Inoculations on Pisang Rastali.		Results.
1	Inoculation just below bud tissue ...	Negative.
2	Inoculation just below bud tissue ...	Negative.
3	Inoculation half way up stem	Negative.
4	Inoculation half way up stem	Negative.
5	Control for 1	Negative.
6	Control for 2	Negative.
7	Control for 3	Negative.
8	Control for 4	Negative.

The above inoculations were made on plants two months old and were carried out on August 12th, 1927. No results up to the present time have been forthcoming.

The following inoculations were carried out on October 18th, 1927, on four months old "Pisang Rastali" plants. The inoculum used was the Fusarium fungus isolated from a "Rastali" rhizome affected with a heart rot obtained from Johore.

		Results.
B. 1	Inoculation just below bud tissue ...	Negative.
2	Inoculation just below bud tissue ...	Negative.
3	Inoculation below ground in bole of rhizome	Positive.
4	Inoculation below ground in bole of rhizome	Negative.
5	Control for 1	Negative.
6	Control for 2	Negative.
7	Control for 3	Negative.
8	Control for 4	Negative.

No. 3 of the above exhibited the first symptoms of disease on January 30th, the incubation period being approximately three and a half months. The external symptoms resembled closely those of Panama disease as described by E. W. Brandes (Phytopathology. 9 Vol. IX). The first symptom was the longitudinal splitting of the outer leaf bases which eventually extended all the way from the rhizome, at the surface of the ground, to where the leaves diverge (see photograph A). Following the longitudinal splitting of the outer leaf sheaths, the outer leaves began to wilt and turn yellow along their edges. This discoloration gradually spread towards the midrib, the colour changing from yellow to dark brown. A few of the outer leaves had dropped over while still quite young. The central leaves remained somewhat stunted soon after the first symptoms of disease appeared.

The plant was taken out about two weeks after the first symptoms of disease were exhibited. Photographs of the diseased plant were taken before being removed (see photograph B) and others were taken in the laboratory showing internal symptoms (see photograph D).

A longitudinal section taken through the above plant shows the discoloured vascular bundles more conspicuously towards the basal portion. The path of the organism through the vascular tissue could be traced from the point of inoculation although this is not very clear in the photograph (see photograph D). The affected vascular bundles were more con-

spicuous in the central tissue of the pseudostem than in the peripheral parts. There was however no central rot within the pseudostem as in the case of the disease in Johore. Several of the roots near the inoculated area were diseased.

The Fungus.

Isolations from the vascular tissue of the inoculated rhizome produced a Fusarium fungus, similar morphologically and culturally, to the fungus originally isolated from the diseased rhizome in Johore.

Stained transverse microtome sections revealed the fungus hyphae massed together within the lumen of the tracheae. The cell walls of the tracheae were also considerably darkened, due possibly to a toxic substance excreted by the fungus. Experiments are in progress at the present time to make comparative tests of the effects of the toxic excretions of the Fusarium fungus from Johore, a type culture of *Fusarium cubense* imported from Manila, and a similar Fusarium fungus associated with what appears to be an outbreak of Panama Disease in Negri Sembilan. These experiments are being carried out by making comparative studies of the effects on bean seedlings immersed in the filtrate from a culture growing in Uschinsky's solution, sterile Uschinsky's solution and sterile water.

Up to the present time in Malaya, only two varieties of bananas are susceptible to what appears to be Panama Disease, these are "Pisang Embun" and "Pisang Rastali."

Following the previous inoculations with the Fusarium fungus, other preliminary inoculations were carried out with the same fungus with a view to finding out whether any of the other common varieties of bananas would be susceptible to the attacks of this organism. Six plants of each variety were used; three plants were used as controls and three were inoculated with the Fusarium fungus. The inoculations were carried out by making wounds below the surface of the ground at the bases of the rhizomes and pouring over a spore suspension of the Fusarium fungus. The varieties experimented with were as follows: Kipas, Talam, Rajah Udang, Kling, Abu, Su-su, Lilin, Masak Hijau, Mas, Mundan, Kelat, Embun and Awak Legor. Of these varieties only two up to the present time have shown symptoms of disease; these are "Pisang Embun" and "Pisang Su-su". The former exhi-

bited the first symptoms of disease three months after inoculation and the latter four months after inoculation.

The outbreak of a banana wilt disease in Negri Sembilan was only recorded during the early part of January this year. This disease more closely resembles Panama Disease as regards both internal and external symptoms than the banana wilt disease in Johore. The four outstanding factors in connection with this disease are—(1) The presence of disease symptoms on mature or nearly full grown plants, (2) The bright yellow discoloration on the margins of the external leaves previous to their drooping over, (3) The longitudinal splitting of the outer leaf sheaths and (4) The preponderance of discoloured vascular bundles in the central parts of the pseudostem and the gradual decrease of affected vascular bundles in the peripheral parts of the pseudostem.

The fungus isolated from the diseased tissue of a plant affected with the above disease was a Fusarium, and closely agrees, morphologically and culturally, with the type species of *Fusarium cubense* (imported from Manila). The cultural characteristics shew various differences as compared with the type species of *Fusarium cubense* and the Fusarium fungus found in Johore. A comparison of the cultural differences of these three Fusaria is as follows:—

Fusarium Fungus from Johore.

On Rice.—Ethyl acetate odour produced in young cultures. Pink discoloration present in young cultures. This discoloration gradually becomes more intensified and red blotches are often present along the sides of the test tube. After about three weeks old the culture turns purple with very little production of aerial myselium.

Bluish purple sporodochial masses are produced in old cultures. The sporodochia produce chlamydospores which are ovoid, thick walled resting spores.

On Potato Plug.—Aerial mycelium produced only in young cultures. Surface of potato turns brown and becomes matted and leathery. The upper portion of the culture is sometimes slightly purple. Yellowish white sporodochia are produced in old cultures. Very old cultures turn white.

On Potato Dextrose Agar Plates.—Patchy purplish discoloration produced in young cultures. Little aerial mycelium present.

Type Culture of Fusarium Cubense From Manila.

On Rice.—No definite odour produced. Slightly redder and more general discoloration produced than in the case of the fungus from Johore. Aerial mycelium remains present for a longer time than in the case of the Johore fungus. Discoloration changes gradually to purple as in the previous case. Bluish purple sporodochia produced in old cultures.

On Potato Plug.—Aerial mycelium remains present for a longer time than in the case of the fungus from Johore. Upper portion of potato turns yellow and becomes somewhat leathery in old cultures. Mycelial growth often present at base. Purplish discoloration often present in streaks. Yellowish white sporodochia produced. Very old cultures turn white.

On potato Dextrose Agar Plates.—Patchy purplish discoloration produced by young colonies. Very little aerial mycelium produced.

Fusarium Fungus from Negri Sembilan.

On Rice.—Benzolic odour present. Mycelium more effuse in young cultures than in the case of the two previous Fusaria. A lighter and more uniform pink discoloration produced as compared with the Johore fungus and type culture. Pink discoloration remains for a longer time and old cultures have a lighter violet discoloration than the two previously described fungi. Yellow sporodochial masses produced in old cultures.

On Potato Plug.—Greater production of mycelium as compared with the Johore fungus and type culture. The sides of the potato plug turn brown and leathery more gradually than in the two previous cases. The production of blue black sclerotia is the chief distinguishing characteristic on this particular medium. The culture at six weeks becomes almost covered with these wart-like excrescences. The sclerotia produce all types of spores within 4-5 days in damp chambers.

On Potato Dextrose Agar Plates.—Considerable cottony white aerial mycelium is produced. No discoloration either in young or old cultures.

From the above it will appear that the Fusarium fungus from Johore and the type culture of *Fusarium cubense* resemble each other more closely than the type culture and the Negri Sembilan culture. Morphologically the three Fusaria are very similar with regard to type of spore production. All three fungi produce the same type of microspore, macrospore and chlamydospore.

A comparison of the spore sizes of these fungi shows small variations when comparing similar types of spores of the three fungi taken at the same age. It is extremely doubtful if spore measurements will ever serve as true distinguishing characteristics.

The preliminary experiments with the fungus from Johore indicated that the fungus was capable of producing a banana wilt disease similar to Panama Disease. Further inoculation experiments have therefore been carried on a larger scale together with comparative inoculation experiments with the Fusarium from Negri Sembilan and with the type culture of Fusarium cubense from Manila.

Two types of inoculations were carried out with each fungus.

(1) Inoculating the rhizome by cutting out a plug and inserting the mycelium. The wounds were afterwards sealed over with wax.

(2) Inoculating the rhizome by making an exposed wound and pouring over a spore suspension of the fungus after rhizome is in the ground.

The varieties used for inoculation purposes were "Pisang Embun" and "Pisang Rastali", also the two wild varieties, *Musa Malaccensis* and *Musa violescens*. The latter two varieties were inoculated with a view to test their susceptibility to disease, so that if they proved non-susceptible, cross pollination with desirable cultivated varieties could be attempted.

The following series of inoculations were carried out on February 11th and 12th on month old suckers.

1.—Sealed up Inoculations on "Embun" Rhizomes.

Four plants used as controls.

Four plants inoculated with type culture of *Fusarium cubense*.

Four plants inoculated with Fusarium fungus from Johore.

2.—Sealed up Inoculations on "Rastali" Rhizomes.

Four plants used as controls.

Four plants inoculated with type culture of *Fusarium cubense.*

Four plants inoculated with Fusarium fungus from Negri Sembilan.

3.—Exposed Inoculations on "Rastali" Rhizomes.

Four plants used as controls.

Four plants inoculated with type culture of *Fusarium cubense.*

Four plants inoculated with Fusarium fungus from Johore.

Four plants inoculated with Fusarium fungus from Negri Sembilan.

4.—Exposed Inoculations on "Embun" Rhizomes.

Four plants used as controls.

Four plants inoculated with type culture of *Fusarium cubense.*

Four plants inoculated with Fusarium fungus from Negri Sembilan.

5.—Sealed up Inoculations on Musa Malaccensis Rhizomes.

Four plants used as controls.

Four plants inoculated with type culture of *Fusarium cubense.*

Four plants inoculated with type culture of *Fusarium cubense.*

In the latter four plants the exposed type of inoculation was used.

6.—Sealed up Inoculations on Musa Violescens Rhizomes.

Three plants used as controls.

Three plants inoculated with type culture of *Fusarium cubense.*

Owing to the above series of inoculations not having had sufficient time for development, the results of these experiments will be published at a later period.

Soil experiments are also in progress at the present time on the diseased area in Negri Sembilan. These experiments are being carried out with a view to finding out whether other varieties of bananas are susceptible to the disease present there, by being attacked directly by the soil organism. Three isolated areas of about an acre each are being used for these experiments, in order to obtain results which would be representative of the land in the whole district. Included in these areas is some jungle land. Results of these experiments will be published later.

Literature Cited.

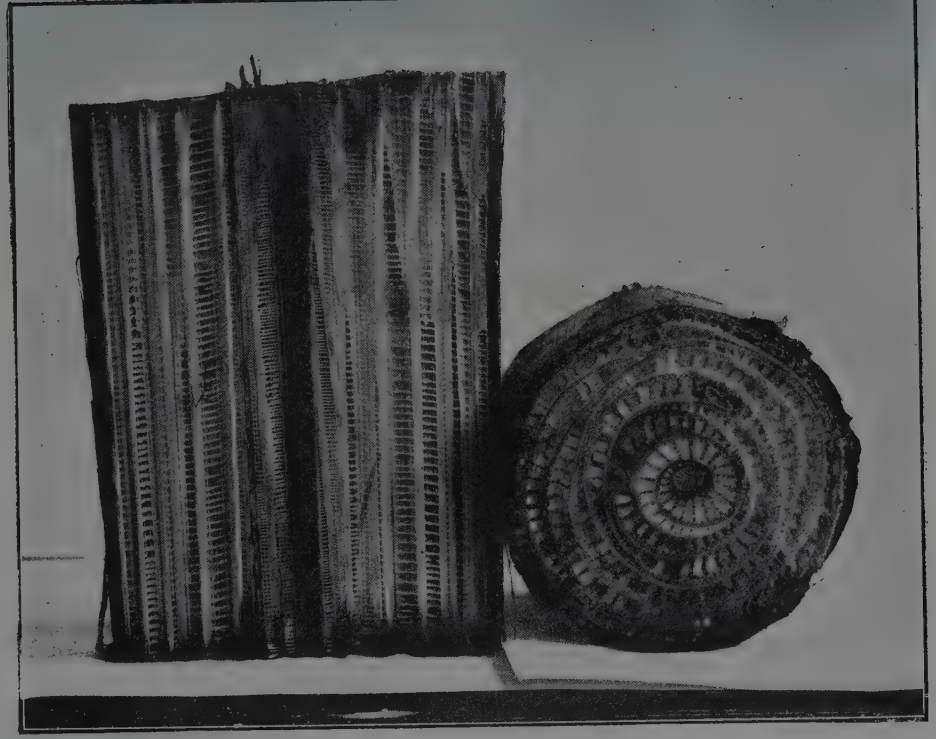
(1) Brandes, E. W.—Phytopathology, Vol. IX, No. 9, Sept. 1919.

(2) Reinking, O. A. & Wollenwebber, H. W.—The Phillippine Journal of Science, Vol. 32, No. 2, Feb. 1927.

Received for publication 28th April, 1928.

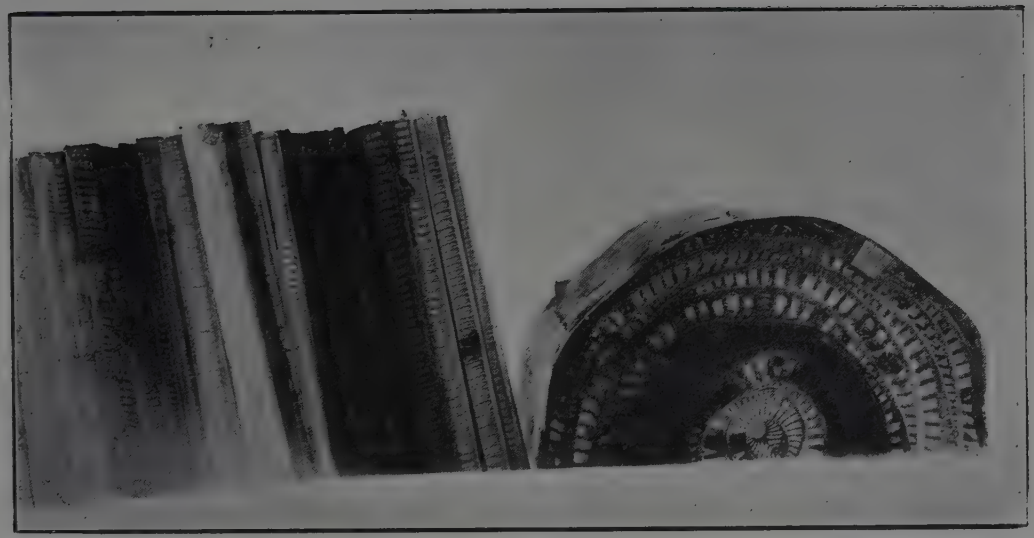
A.—Pisang Restali inoculated with Fusarium fungus from Johore. Longitudinal splitting of the outer leaf sheaths is shewn.

B.—Pisang Restali on extreme right the same as in A. The drooping of the external leaves and the stunted appearance of central leaves is seen. The two groups on the left are controls.



C.—Photograph showing longitudinal and transverse sections of pseudo-stem of "Pisang Restali" affected with a heart rot in Johore. Fusarium fungus isolated from this type of disease.

D.—Longitudinal section of inoculated Pisang Restali. The strands of affected vascular tissue are seen on right.



E.—Transverse and longitudinal sections of diseased Pisang Restali plant from Johore. Bacteria were isolated from the gummy exudate of plants affected with this type of disease.

Brief Notes on Agricultural Conditions on the East Coast of Malaya.

H. W. Jack.

Recently the writer has been privileged to visit some of Unfederated States on the east coast of Malaya, and these notes are merely intended as a brief record of impressions of the general agricultural conditions found there.

Climatic conditions, of course, differ from those prevailing in the western states in that they are influenced greatly by the south-east monsoon and seasonal changes are tolerably regular in their incidence within limits. Thus, there is a fairly well defined wet period extending from September to early February during which some 85 inches of rain may be expected, followed by an equally definite dry season which normally covers the period March to August when there is an average monthly rainfall of 5.5 inches. The monsoon rains are most abundant in the months of November and December as a rule, though little reliance can be placed on the actual incidence of the heavier rains and consequently floods are of frequent occurrence but of brief duration—thanks to the numerous river channels and the general flatness of the coastal lands.

The population in the east coast states is sparce, amounting to less than 50 persons per square mile, and the inhabited areas are almost entirely confined to the fishing villages which are found at intervals along the seashore and to the lower reaches of the rivers where they cut their beds through the flat sandy coastal belt which is typical of the country.

The combined action of the sea, floods in the rivers, and the monsoon winds has created a sand bank which varies in width from about one chain to perhaps half-a-mile or more, and which stretches up and down the coast dividing the sea from the inland lowlying areas. These low-lying areas extend inland to as much as 4 or 5 miles and are, in places, useful for padi cultivation and kampong; sometimes they are too deep and too swampy for cultivation and sometimes they are very narrow and deep forming wide natural canals and mingling with the river channels.

These sandy flats gradually merge into gently undulating light loamy areas which in turn become hills several miles distant from the sea. The hinter land is mountainous and quite unexplored save by the Sakai.

The flattish formation of the inhabited coastal belt, the abundant rainfall during the wet season and the general distribution of the population are factors which of themselves indicate that the predominant agricultural feature of the country is padi cultivation which extends southwards from the boundary of Siam, wherever there is any settlement.

A striking characteristic of the inhabited lands is their general openness and complete absence of lallang which so quickly smothers all open land on the west coast. This openness is a direct result of the form of agriculture which has probably held sway for hundreds of years. The rice fields are well ploughed, the bullocks feeding on the grasses which spring up in the kampongs during the padi season and grazing the rice fields during the dry season so that lallang is never permitted to reach the flowering stage or even to grow at all.

Moreover, rubber which so densely covers the cultivated areas of the west coast is scarcely known, all the crops which are grown being annuals with the exception of coconuts and arecanuts, which do not spoil the gladelike appearance of the kampongs.

The soils of the east coast are more varied in texture than those of the west coast states. The foothills afford medium clays and porous loams, the flat lands are composed of light and sandy loams overlying sandy beds which make drainage very good, and the lowlying areas consist of a thin layer of loamy clay lying on a sand bottom.

Practically no heavy clay soils were noticed except in small depressions, the bulk of the soils being readily classed as light sandy loam, which is easily workable but which, without plenty of tillage, abundant water supply and some manuring, is precarious as a crop producer.

The necessity for thorough tillage coupled with the dependence of the Malays on their rice crops as their main source of food, is reflected in the physique of the people for they appear to be more robust than their fellows on the west

coast who are more fortunate in having easier means of existence and in not being isolated for spells by monsoon weather.

During the incidence of the monsoon, shipping is sometimes entirely suspended at many of the ports of call for several weeks at a time so that the Malays who seem to accept floods in a wonderfully quiet and philosophical manner, have to fend for themselves, if their previous rice crop has not been good and consequently a bigger variety of food crops is found in the kampong than is generally noticeable on the western coast.

The question of transport does not appear very difficult on the east coast though much development of rail and road traffic is desirable. Already the railway, passable roads, and the river connect up Kelantan headquarters with the up-country districts and the port of Tumpat. In Trengganu there is no railway so far and roads are only beginning to be useful but the sixteen rivers which run from the hills to the sea make transport easy except possibly after a long dry spell; moreover, the bulk of the population at present lives within a mile or two of the seashore which is some 200 miles long and which is well served by various steamers and launches. At present Trengganu is more or less isolated from the outside world in monsoon weather except for the northern districts which are in road connection with Kelantan and thence with the railway.

Security of land tenure which was previously doubtful and dependent on the whims of the ruling Malays is now assured since British Advisors have been installed though for lack of survey, little land is yet available to the capitalist planter or miner.

The comparative absence of capitalist pioneers or large industries is very regrettable because the people have little in the nature of wage earning facilities to tide them over bad rice seasons and to enable them to enjoy a better standard of living. This condition in the economic life of the Malay is less striking in Kelantan where the railway development combined with road construction and an influx of rubber planters, have been instrumental in affording daily employment for thousands of eager workers—but there is not yet enough scope in that direction.

Land is available in abundance or rather will be as soon as surveys can be prepared but there is no very urgent demand for land except in a few districts, since population is so small and tends to cling to the towns where entertainments and other luxuries are more available than in the kampongs. Customary normadic methods of extracting a living from the soil, together with a surplus of land have created an extensive form of cultivation, which is wasteful of land and of energy but in the more settled areas intensive farming is gradually displacing the old extensive methods with many attendant adantages. The comparative absence of the Indian moneylender is noteable, though Mohamedan Indian merchants are plentiful and doubtless their profits are sufficiently satisfactory. The small holder on the average has a trying time since his main food crop is so very dependent on the whims of the weather and his surplus padi or other money crops get poor prices for various reasons.

There are no set standards for weights or measures in many areas, neither is there machinery to enable any set standards to be enforced so that the wily and unscrupulous buyer trades too much on the ignorance of his customers and on their improvidence when food supplies are low or when they become ensnared by the prevalent vice of gambling. Neither is there any indication of cooperation amongst the crop producers who are sadly fleeced by middlemen, though apparently the merchants combine sufficiently to "rig the markets" at suitable times with distinct pecuniary advantage to themselves.

The saving grace of the country is the enforced industry of the cultivator which has become habitual, for life is hard and the means of existence demand strenuous effort and exposure to risk (fishing)—two factors which tend to develope the finest type of agriculturists so that there should be a big future looming ahead for our eastern states especially if agriculture is fostered and developed as the mainstay of the country.

Received for Publication 18th April, 1928.